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Rules for Distinguishing Toxicants That Cause Type I and Type II Narcosis Syndromes

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Narcosis is a nonspecific reversible state of arrested activity of protoplasmic structures caused by a wide variety of organic chemicals. The vast majority of industrial organic chemicals can be characterized by a baseline structure-toxicity relationship as developed for diverse aquatic organisms, using only the *n*-octanol/water partition coefficient as a descriptor. There are, however, many apparent narcotic chemicals that are more toxic than baseline narcosis predicts. Some of these chemicals have been distinguished as polar narcotics. Joint toxic theory and isobole diagrams were used to show that chemicals strictly additive with phenol were generally more toxic than predicted by narcosis I models and characterized by a different mode of action called narcosis II syndrome. This type of toxicity is exemplified by certain amides, amines, phenols, and nitrogen heterocycles. Evidence is provided that suggests that narcosis II syndrome may result from the presence of a strong hydrogen bonding group on the molecule, and narcosis I syndrome results from hydrophobic bonding of the chemical to enzymes and/or membranes. This shift in toxic action is apparently indistinguishable for narcotic chemicals with log P greater than about 2.7. General rules for selecting the appropriate models are proposed.

Introduction

Narcosis is a reversible state of arrested activity of protoplasmic structures caused by a wide variety of organic chemicals. Veith et al. (*1*) demonstrated that this nonspecific mode of action was responsible for lethality in the fathead minnow (*Pimephales promelas*) for many alcohols, ketones, ethers, alkyl halides, and benzene derivatives. The structure-toxicity relationships developed by both Konemann (*2*) and Veith et al. (*1*) are very similar and accurately estimate the LC_{50} of nonreactive, nonpolar chemicals for a wide variety of aquatic organisms (*3*) using only the *n*-octanol/water partition coefficient (log P). The equations presented (*1,2*) have become known as baseline toxicity models that predict the toxicity of chemicals that act through the nonspecific mechanism of narcosis. In general, more specific mechanisms produce greater toxicity than baseline narcosis.

We are attempting to develop structure-toxicity relationships for other mechanisms of lethality and to determine the structural requirements of the chemicals that act through a given mechanism. Since detailed mechanistic studies for all chemicals are not possible, we have clustered chemicals using fish acute toxicity syndromes (FATS) (*4*) based on physiological and behavioral symptoms during the test. Baseline narcosis is characterized by progressive lethargy, unconsciousness, and death without any specific sustained symptoms such as hyperventilation, erratic or convulsive swimming, or hemorrhage. Despite the fact that the vast majority of nonreactive industrial chemicals produce symptoms of baseline narcosis, we found many apparent narcotic chemicals to be substantially more toxic than our initial baseline narcotic structure-toxicity relationship predicts.

Ferguson (*5*) distinguished the more toxic narcotics as polar narcotics because these chemicals were more soluble in water. Ljublina and Filov (*6*) also distinguished the more toxic narcotics on a basis of greater water solubility. Kamlet et al. (*7*) recently showed that the increased toxicity of these narcotics was correlated with greater dipolarity and/or hydrogen bond donor acidity of these structures. Kamlet et al. (*7*) proposed that, if the mechanisms underlying these two narcosis syndromes were the same, a single uniform structure-toxicity relationship for narcosis could be used to predict lethality. Using the FATS methodology mentioned above, however, the toxic responses of nonpolar and polar (aniline and phenol derivatives) narcotics are clearly distinguishable (*8*).

Franks and Lieb (*9,10*) provide convincing arguments that the depression of activity by general anesthetics occurs through competitive inhibition of key target enzymes. Hydrophobic binding in pockets of specific en-

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zymes in the central nervous system (yet to be identified) has been proposed as the most likely mechanism leading to inhibition by nonpolar narcotics. Broderius and Kahl (*11*) used joint toxicity theory and isobole diagrams to show that nonpolar narcotics are strictly additive when tested as mixtures. The condition of strict additivity is necessary for the mechanism to be considered similar for different chemicals. In this paper, we review the evidence for dissimilarity of the underlying mechanisms of nonpolar and polar narcosis. We have defined polar narcosis as the narcosis II syndrome and proposed a structure-toxicity relationship for the narcosis II toxicants (*12*).

Materials and Methods

This work extends the use of joint toxicity tests to discriminate between narcosis I and narcosis II chemicals. Toxicity tests were conducted according to standard procedures (*13*) and have been described in detail previously (*11*). Briefly, juvenile fathead minnows (*Pimephales promelas*) were placed in continuous-flow diluters having five treatment concentrations and a control for each test. Mortalities were recorded daily, and the estimated median lethal concentration (LC_{50}) was determined after 96 hr. Binary mixtures of chemicals were tested at ratios of 5:0, 4:1, 2:1, 1:1, 1:2, 1:4, and 0:5. The 96-hr LC_{50}s of these binary mixtures were used to construct isoboles (*14,15*) of joint toxic action (Fig. 1). The procedures used to analyze results by concentration or response addition models are those proposed by Finney (*17*) and Anderson and Weber (*18*).

The original work of Veith et al. (*1*) used linear aliphatic alcohols as model nonpolar narcotics, or narcosis I toxicants. Broderius and Kahl (*11*) demonstrated the use of *n*-octanol as a reference narcosis I toxicant to test for strict additivity with other toxicants. It was proposed that chemicals which were strictly additive with octanol could be considered narcosis I chemicals and were accurately modeled by the QSARs of Konemann (*2*) and Veith et al. (*1*). For this study, we selected phenol as a reference narcosis II toxicant for all joint toxicity tests. The symptoms of fish exposed to phenol are consistent with narcosis II (*8*) and the toxicity is generally greater than predicted by the narcosis I model.

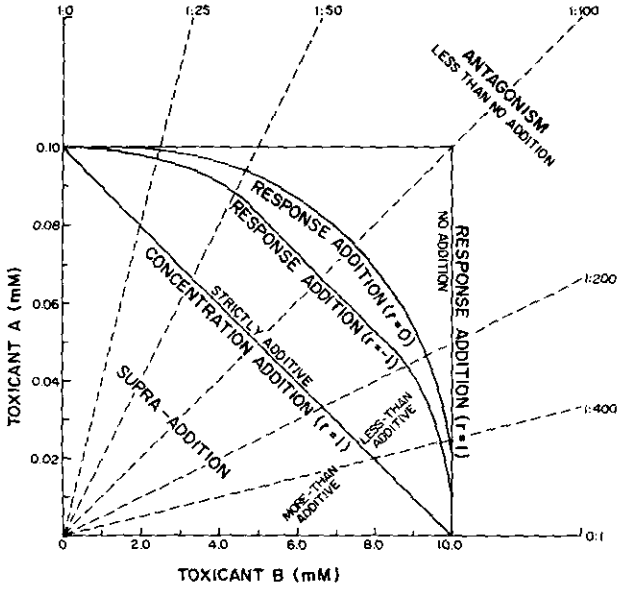

FIGURE 1. Isobole diagram depicting various types of lethal responses for the joint action of two toxicants displaying parallel concentration-response curves. After Muska and Weber (*16*).

Results and Discussion

The toxicity of some amides, amines, phenols, and nitrogen heterocycles are underestimated by the baseline narcosis QSAR presented by Veith et al. (*1*). Assuming that this QSAR is the narcosis I syndrome, we proposed that these polar chemicals produce narcosis through a different mode of action which we call the narcosis II syndrome. We determined that phenol or aniline are not strictly additive in their joint toxic response with octanol, our reference narcosis I chemical. These results clearly suggest a second mechanism. We tested more than 50 polar chemicals for strict additivity with phenol. Compounds that demonstrate strict additivity with phenol are presented in Table 1, together with the toxicity of the chemicals. Table 1 shows that a wide variety of substituted phenols and primary amines are strictly additive with phenol. This evidence strongly supports the use of a separate structure-toxicity relationship for these polar chemicals.

The joint toxicity studies revealed several important factors with respect to the structural requirements of narcosis II. Five of the phenols and three anilines were strictly additive with both octanol and phenol (Table 1). All of these chemicals except ethylaniline have log P values greater than 2.7, whereas those not additive have log P values less than 2.7. Two compounds with log P greater than 2.7, 1-naphthol and 4-chloro-3-methylphenol, were not tested in combination with 1-octanol. These data are consistent with the concept that the narcosis II syndrome may result from the presence of a strong hydrogen bonding group on the molecule and the narcosis I syndrome results from hydrophobic bonding of the chemical to enzymes and/or membranes. As the log P of the chemicals increases, the relative contribution of hydrogen bonding to the toxicity seemingly decreases in favour of the hydrophobic bonding of the toxicant. For narcotic chemicals with a log P of 2.7 or greater, it appears that the influence of hydrophobic bonding is equal to or greater than hydrogen bonding. Not only would either QSAR model estimate the toxicity equally well for these lipophilic chemicals, but also the underlying mechanisms of enzyme inhibition is seemingly indistinguishable.

We have noted that all of the chemicals in Table 1 are weakly acidic or basic. Strongly acidic phenols and cer-

Table 1. Chemicals with strictly additive joint toxicity with phenol.

Compound	CAS[a] number	Log M 96-hr LC_{50}	Log P[b]
Amides			
4-Nitrobenzamide	619-80-7	− 3.10	0.82 (m)
Primary aliphatic amines			
1,2-Diaminopropane	78-90-0	− 1.77	− 0.91
Primary aromatic amines			
Aniline	62-53-3	− 2.84	0.90 (m)
4-Nitroaniline	100-01-6	− 3.04	1.31
4-Chloroaniline	106-47-8	− 3.59	1.83 (m)
2-Chloroaniline	95-51-2	− 4.35	1.90 (m)
4-Ethylaniline[c]	589-16-2	− 3.22	1.96 (m)
2-Chloro-4-nitroaniline	121-87-9	− 3.93	2.17
4-Bromoaniline	106-40-1	− 3.56	2.26 (m)
2-Chloro-4-methylaniline	615-65-6	− 3.59	2.58
3,4-Dichloroaniline[c]	95-76-1	− 4.33	2.69 (m)
2,3,4-Trichloroaniline[c]	634-67-3	− 4.73	3.33 (m)
Substituted phenols			
Catechol	120-80-9	− 4.08	0.81
4-Amino-2-nitrophenol	119-34-6	− 3.63	0.96
Phenol	108-95-2	− 3.59	1.46 (m)
3-Methoxyphenol	150-19-6	− 3.22	1.58 (m)
4-Nitrophenol	100-02-7	− 3.53[d]	1.91 (m)
2,4-Dimethylphenol	105-67-9	− 3.87[d]	2.30 (m)
4-Ethylphenol	123-07-9	− 4.07	2.58 (m)
1-Naphthol	90-15-3	− 4.49	2.84 (m)
4-Propylphenol[c]	645-56-7	− 4.09	3.18
2-Phenylphenol[c]	90-43-7	− 4.44	3.36
p-Phenoxyphenol[c]	831-82-3	− 4.58	3.75
p-tert-pentylphenol[c]	80-46-6	− 4.80	3.98
Halogenated phenols			
2-Chlorophenol	95-57-8	− 4.14	2.15 (m)
2,4-Dichlorophenol[c]	120-83-2	− 4.32[d]	2.92 (m)
4-Chloro-3-methylphenol	59-50-7	− 4.40	3.10 (m)
Pyridines			
4-Acetylpyridine	1122-54-9	− 2.86	0.48 (m)
2-Cyanopyridine	100-70-9	− 2.16	0.50 (m)
Pyridine	110-86-1	− 2.90	0.65 (m)
6-Chloro-2-pyridinol	16879-02-0	− 2.78	1.78

[a] Chemical Abstract Service registry number.
[b] Computer calculated by CLOGP version 3.4 software or a measured value (m) retrieved from STARLIST (*23*).
[c] Chemicals also strictly additive with octanol.
[d] From Holcombe et al. (*24*).

tain anilines are much more toxic than either the narcosis I or narcosis II QSAR would predict, and the toxicity syndrome is clearly not that of narcosis. Rules for discriminating these compounds must be consistent for differentiating an additional toxicity mechanism. Chemicals that do not elicit the narcosis II syndrome and are less than strictly additive with phenol and octanol include phenols or anilines with two or more nitro substituents, or four or more ring substituted halogens. These are more toxic than is estimated from narcosis QSAR models and are likely oxidative phosphorylase uncouplers. This mechanism and associated QSAR will be discussed elsewhere.

The overall results are summarized in Figures 2 through 4. Figure 2 presents the variation in toxicity of narcosis I chemicals with log P from three reference systems. These data establish the narcosis I structure-toxicity relationship. Figure 3 presents a group of narcosis II chemicals that are strictly additive with phenol but not octanol. The line for the narcosis II structure-toxicity relationship is summarized by Veith and Broderius (*12*) as follows:

$$\log LC_{50} = -0.65\ (\pm 0.07) \log P - 2.29\ (\pm 0.22)$$
$$n = 39,\ r^2 = 0.90$$

The chemicals that are strictly additive with octanol and phenol are presented in Figure 4.

Summary of Narcosis Selection Rules

Narcosis is thought to be a reversible and rather nonspecific mode of toxic action. Careful examination of the different symptoms caused by a wide variety of narcotic chemicals suggests the possibility that there are numerous mechanisms of narcosis. Creating structure-toxicity relationships for each may be impossible; nonetheless, we can establish QSARs for major groups and then develop general rules for selecting the appropriate model.

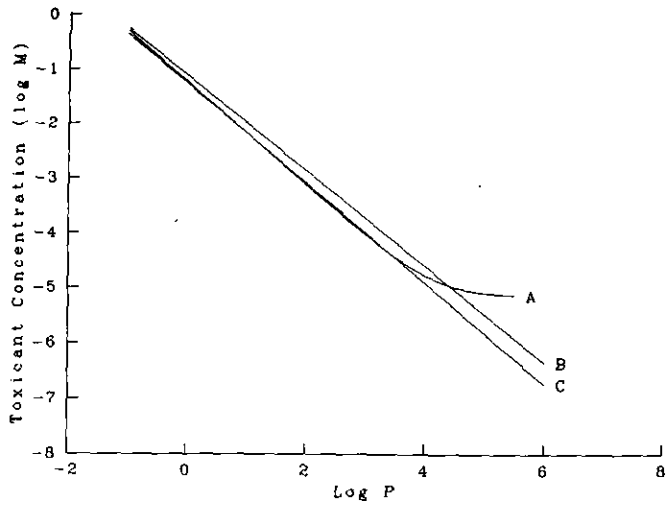

FIGURE 2. Acute toxicity QSAR model regression lines for narcosis I industrial organic chemicals as determined by (*A*) Veith et al. (*1*) for the fathead minnow (96-hr LC_{50}); (*B*) Konemann (*2*) for the guppy (7- or 14-day LC_{50}); and (*C*) Hermens et al. (*19*) for *Daphnia magna* (48-hr IC_{50}).

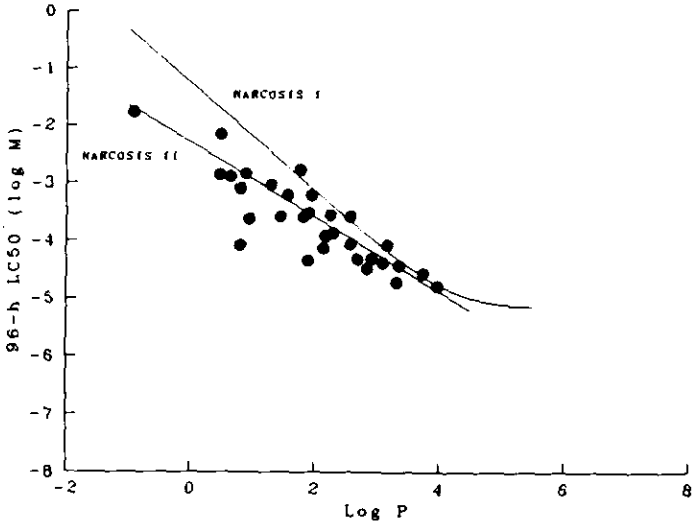

FIGURE 3. Acute toxicity to juvenile fathead minnows of chemicals that are strictly additive in their joint toxicity with phenol. Narcosis I and II model lines are from Veith et al. (*1*) and Veith and Broderius (*12*), respectively.

First, the nonspecific nature of narcosis means that chemicals that meet the structural requirements of specific modes of action should be excluded from narcosis QSARs. Narcosis is appropriate only for nonreactive toxicants. Excluded are chemicals which irreversibly bind to natural products through electrophilic reactions (*20*), are metabolically activated to electrophiles, chemicals such as aldehydes which can form Schiff-bases with amino groups, and Michael-type acceptors.

Kamlet et al. (*21*) found that amines and carboxylic acids that are strong proton-transfer acids and bases do not conform to narcosis models of nonelectrolytes. We have found that the aquatic toxicity of many alkyl amines and tertiary anilines can be estimated using the

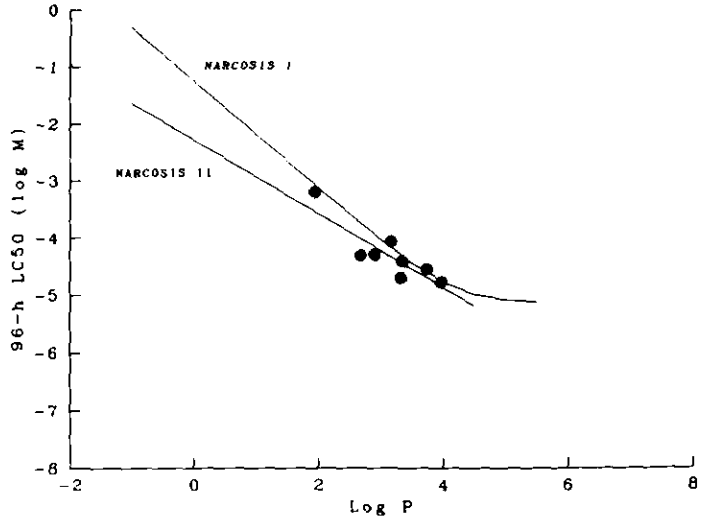

FIGURE 4. Acute toxicity to juvenile fathead minnows of chemicals that are strictly additive in their joint toxicity with either 1-octanol or phenol. Narcosis I and II model lines are from Veith et al. (*1*) and Veith and Broderius (*12*), respectively.

narcosis I QSAR. The toxicity of these chemicals is not additive with phenol. Substituents on the nitrogen of amines decrease the polarity of the chemicals and shift the toxicity syndrome from narcosis II to narcosis I.

Carboxylic esters have presented a special problem in the development of structure-activity relations. The literature is replete with examples of anomalous behavior of esters. Kamlet et al. (*21*) excluded esters from QSARs for narcosis because they rationalized that esters were subject to *in vivo* hydrolysis. The increase in toxicity of esters over narcosis I chemicals is consistent with and correlated to the alkaline hydrolysis rate constant in water, even though hydrolysis is likely to be a detoxification mechanism. Veith et al. (*22*) reported that esters have similar symptoms to narcosis I chemicals but are more toxic. The QSAR reported for esters was

$$\log LC_{50} = -0.535 \log P - 2.75$$
$$n = 29,\ r^2 = 0.828$$

which is nearly identical to the narcosis II QSAR presented earlier. However, in joint action studies, monoesters were strictly additive with octanol, which suggests that the narcosis I QSAR might be improved by including the dipolarity/polarizability term, π^*, proposed by Kamlet et al. (*21*) to account for increased toxicity. Diesters were found to be less than additive with both octanol and phenol and their toxicity is generally greater than that estimated from the narcosis I QSAR. Until the underlying mechanism of ester toxicity is understood, we recommend using the QSAR for ester narcosis.

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